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Assessing tumor cytoarchitecture using multi-echo DSC-MRI derived measures of the Transverse Relaxivity at Tracer Equilibrium (*TRATE*)

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Abstract

Purpose—In brain tumor dynamic susceptibility contrast (DSC)-MRI studies, multi-echo acquisition methods are used to quantify the dynamic changes in T_1 and T_2^* that occur when contrast agent (CA) extravasates. Such methods also enable the estimation of the effective tissue CA transverse relaxivity. The goal of this study was to evaluate the sensitivity of the Transverse Relaxivity at Tracer Equilibrium (*TRATE*) to tumor cytoarchitecture.

Theory and Methods—Computational and *in vitro* studies were used to evaluate the biophysical basis of *TRATE*. In 9L, C6 and human brain tumors, *TRATE*, the apparent diffusion coefficient (*ADC*), the CA transfer constant (K^{trans}), the extravascular extracellular volume fraction (v_e) and histological data were compared.

Results—Simulations and *in vitro* results indicate that *TRATE* is highly sensitive to variations in cellular properties such as cell size and density. The histologic cell density and *TRATE* values were significantly higher in 9L tumors as compared to C6 tumors. In animal and human tumors, a voxel-wise comparison of *TRATE* with *ADC*, v_e , and K^{trans} maps showed low spatial correlation.

Conclusion—The assessment of *TRATE* is clinically feasible and its sensitivity to tissue cytoarchitectural features not present in other imaging methods indicate that it could potentially serve as a unique structural signature or “trait” of cancer.

Keywords

dynamic susceptibility contrast; transverse relaxation; vascular structures; cellular structures; contrast agent leakage; contrast agent equilibrium

Introduction

The assessment of perfusion parameters using DSC-MRI has proven useful for assessing tumor grade (1-7) and treatment response (3,8-12). In normal brain regions, with an intact blood-brain barrier (BBB), the biophysical basis of the acquired DSC-MRI signal is well characterized, primarily reflecting the underlying contrast agent (CA) kinetics and vascular geometry (13). When the BBB is disrupted, small molecular weight contrast agents (CA), such as Gadolinium- Diethylenetriaminepentaacetic Acid (Gd-DTPA) may extravasate, leading to dynamic changes in the tissue T_1 , T_2 and T_2^* relaxation times. In such cases, DSC-MRI signals are more complex and depend on hemodynamics, vascular geometry and permeability, the extravascular microstructure and the applied pulse sequence (14-16). When DSC-MRI signals are acquired and processed without consideration of CA extravasation, the derived hemodynamic parameters are well known to be unreliable (4,5,17). Several techniques have been proposed and employed to correct for T_1 and T_2^* leakage effects (14,17-22).

In addition to improving the reliability of DSC-MRI data acquired in the presence of CA leakage, there is an increased interest in leveraging the temporal characteristics of such signals to estimate additional biological information. Pharmacokinetic modeling of DSC-MRI signals in brain tumors has been used to extract CA extravasation rate constants (e.g. K^{trans}) and the extravascular extracellular volume fraction (v_e) (18,22-26). The appearance of leakage effects on DSC-MRI signals, whether they are predominantly T_1 or T_2^* -based, has been characterized by the Percent Signal Recovery (PSR), a parameter potentially capable of differentiating between gliomas, metastasis and lymphomas (27). Recent studies have shown that CA leakage-induced T_2^* effects are dependent upon the extravascular geometry (14-16,28,29) and by quantifying these effects, new imaging biomarkers may be derived (16). Once validated, such parameters could improve the characterization of brain tumors.

Currently, multi-echo DSC-MRI, along with a pre-contrast T_1 map, is used to characterize T_1 and T_2^* leakage effects, as this enables their simultaneous separation and quantification (15,16,22,24). The dynamic T_1 change enables estimation of the tissue CA concentration. Combining the CA concentration with the dynamic T_2^* changes enables the assessment of the tissue CA transverse (T_2^*) relaxivity. Recently, Sourbron et al used this approach in colorectal cancer xenografts to evaluate the vascular and extravascular CA relaxivity, and found that it provided supplementary information on tumor microstructure that is distinct from traditional compartmental volume fraction measurements (16).

In this study, we aimed to evaluate whether multi-echo DSC-MRI derived measures of the Transverse Relaxivity at Tracer Equilibrium (*TRATE*) may be used to evaluate brain tumor cytoarchitecture. To validate the sensitivity of *TRATE* to tumor cellular features we employ a 3D biophysical simulations and compare its characteristics *in vitro* and in two animal brain tumor models that are known to have histologically different cellular properties. We also present initial results of *TRATE* data in a cohort of glioma patients. The *TRATE* maps are spatially compared to parameters traditionally derived from DSC-MRI, Dynamic Contrast Enhanced (DCE)-MRI and Diffusion Weighted Imaging (DWI) in order to preliminarily assess its potential to provide unique sensitivity to microstructural features not assessed with these techniques.

Method

Theory

When CA extravasation effects are present, the tissue transverse relaxation rate depends on both dipole-dipole microscopic interactions between the CA and water protons and mesoscopic effects due to magnetic field perturbation induced by CA compartmentalization within vascular walls and around cells. Models have been previously proposed (14-16,18), that incorporate both microscopic and mesoscopic contributions to the measured transverse relaxation rate change (R_2^*):

$$\Delta R_2^* = r_2 (v_e C_e + v_p C_p) + r_{2p}^* v_p (v_e |C_p - C_e| + v_i C_p) + r_{2e}^* v_e v_i C_e \quad [1]$$

Here v_e , v_i and v_p denote volume fractions of the extravascular extracellular space (EES), extravascular intracellular space (EIS) and vascular space, respectively. C_e and C_p represent the CA concentration of the EES and the vascular space, r_{2p}^* and r_{2e}^* are the effective T_2^* relaxivities of CA compartmentalized within the vascular space and EES, and r_2 is the CA T_2 relaxivity.

This model expresses the tissue CA concentration (C_t) as the sum of individual compartment CA concentrations weighted by the corresponding volume fractions ($C_t = v_e C_e + v_p C_p$), and assumes that the CA does not penetrate into cells and a fast water exchange process. At time points well past the first pass of CA, when C_e and C_p are approximately equivalent, equation (1) can be written as:

$$\Delta R_2^* = \left\{ r_2 + \frac{v_i}{v_p + v_e} (v_p r_{2p}^* + v_e r_{2e}^*) \right\} C_t \quad [2]$$

The first bracketed term in equation (2) can be considered the effective tissue transverse relaxivity at CA equilibrium ($r_{2t,eq}^*$) and will be termed the transverse relaxivity at tracer equilibrium (*TRATE*) hereafter. With this definition, equation (2) simplifies to:

$$\Delta R_2^* = r_{2t,eq}^* \times C_t \quad [3]$$

With multi-echo DSC-MRI data and a pre-contrast T_1 map the tissue CA concentration and the R_2^* can be computed using the extracted T_1 and T_2^* changes, respectively, thereby enabling the computation of *TRATE*.

Simulations

To investigate the dependence of *TRATE* on tissue vascular and cellular features, simulated realistic 3D tissue structures were created using randomly packed ellipsoids around fractal tree based vascular networks (29). Vascular networks that mimic the architecture found in normal and tumor tissue were computed using a fractal tree approach (30,31). To achieve cell densities and cellular orientation heterogeneity that approximate those found *in vivo*, we used randomly packed ellipsoids to model the extravascular space (32). Magnetic field perturbations induced by susceptibility variations between the simulated tissue compartments and the associated gradient echo transverse relaxation rates were computed using the Finite Perturber Finite Difference Method (FPFDM) (29). In addition to a binary 3D matrix that defines the tissue structure, the FPFDM requires, as input, the static magnetic field strength, the susceptibility difference between simulated tissue compartments, the water diffusion coefficient, and pulse sequence parameters.

For CA concentration levels corresponding to tracer equilibrium, the slope of the computed R_2^* versus C_I was used to estimate *TRATE*. To investigate the relative contribution of the vascular and extravascular features to *TRATE* measurements, the signal relaxation was computed using tissue models with fixed cellular features and variable vascular volume fractions. Furthermore, to evaluate the dependence of R_2^* and *TRATE* on cellular features, such as cell volume fraction and cell size, the vascular volume fraction was kept fixed while these features were systematically varied.

Unless mentioned otherwise, all simulation studies were carried out using the following input parameters. All input tissue structures consisted of a $(0.25 \text{ mm})^3$ 3D volume sampled with 256^3 simulation grids. The restricted water diffusion coefficient (D) was set to $1.3 \times 10^{-3} \text{ mm}^2/\text{s}$ (33) and clinically relevant TE and B_0 values of 40 ms and 3 T were chosen. The susceptibility difference between compartments was calculated using $\chi = \chi_m \cdot [\text{CA}]$, where $[\text{CA}]$ is compartmental CA concentration and χ_m is the CA molar susceptibility ($0.027 \times 10^{-6} \text{ mM}^{-1}$) (34). While the susceptibility-induced relaxation was computed using the FPFDM, the relatively small effects of microscopic transverse relaxation were calculated and included using the product of C_I and the Gd-DTPA T_2 relaxivity ($r_2 = 4.5 \text{ mM}^{-1} \text{ s}^{-1}$ (35)). All simulations were performed in Matlab (Mathworks, Natick, MA).

Animal studies

Measures of *TRATE* were compared in two rodent brain tumor models, the C6 glioblastoma and 9L gliosarcoma. Seven male Wistar rats and nine male Fischer rats (Harlan, Indianapolis, IN, USA) were inoculated with 1×10^5 C6 and 1×10^5 9L cells (American Type Culture Collection, Manassas, VA, USA), respectively. Prior to all surgical and imaging procedures, animals were immobilized in a stereotactic head holder. Anesthesia was induced via a 5%/95% isoflurane/oxygen mixture and maintained via a 2%/95% isoflurane/oxygen mixture.

All experiments were carried out 14–16 days after tumor inoculation and adhered to our institution's animal care and use committee policies. Scans were conducted at 4.7T (Agilent) with a Doty shielded Litz coil (38 mm ID). A pre-contrast T_1 (T_{10}) map was obtained using a gradient-echo based multiple flip angle approach with the following parameters: TR = 200 ms, TE = 2 ms, FOV = (40 mm)², slice thickness (ST) = 2.0 mm, matrix = 64², five flip angles (FA) (ranging from 12° to 60°), and 4 excitations. A multi-echo fast low angle shot sequence was used to acquire DSC-MRI data with a temporal resolution of one image per second for a total duration of 17 minutes with: TR=15.625 ms, TE1/TE2=4/8 ms, FOV=(40 mm)², ST=2.0 mm, FA=9°, and matrix=64². After the acquisition of 60 baseline images, a bolus of Gd-DTPA (0.2 mmol kg⁻¹ per body weight) was intravenously delivered using a power injector at an infusion rate of 2.4 ml/min. As described above, the multi-echo DSC-MRI sequence was used to quantify T_1 and T_2^* time series. The pre-contrast T_1 maps were combined with the T_1 -weighted time series to derive R_1 (36). The last two minutes of the R_1 and R_2^* time series data were then used to compute *TRATE* using:

$$C_t = \Delta R_1 / r_1, \quad \text{and} \quad r_{2t,eq}^* = \Delta R_2^* / C_t \quad [4]$$

where r_1 is the Gd-DTPA T_1 relaxivity (3.9 mM⁻¹ s⁻¹) (35).

The reference region pharmacokinetic model was applied to the R_1 time series in order to compute the DCE-MRI parameters, v_e and K^{trans} (24,37). Furthermore, DWI was performed to estimate *ADC* maps using a fast spin echo sequence with diffusion gradients applied along three directions. All diffusion data were obtained using TR=2000 ms, TE=30 ms, FOV=(40 mm)², ST=1.0 mm, matrix=64², separation of diffusion gradients = 15 ms, effective diffusion pulse duration $\delta = 3$ ms, diffusion time ($\delta - \delta/3$) = 14 ms, and five diffusion gradient amplitudes varied from 0 to 33 G/cm ($b = 0$ to 1000 s/mm²).

For region of interest (ROI) analysis the tumor ROIs were manually defined on the dynamic contrast enhanced images. All voxels exhibiting contrast enhancement on the last image of the dynamic time series were included in the ROI. Same-sized normal tissue ROIs were selected in contralateral brain in the same slices as that used for the tumor ROIs.

To evaluate tumor cellularity, all animals were sacrificed after the MRI exams, the tumor tissue dissected and fixed in 10% formalin, cut into 5- μ m sections and stained with hematoxylin and eosin (H&E). The H&E slides were digitally scanned and analyzed to quantify cell nuclei count using an Ariol SL-50 automated scanning microscope. A manual threshold approach is used to discern between the intra- and extra-cellular spaces to estimate cell volume fraction. The cell nuclei count along with an assumed circular cell shape was used to obtain estimates of the mean cell size for each tumor cell line.

In addition to the animals studies described above *in vitro* cell studies were also used to investigate the biophysical basis of *TRATE* as described in Supporting Figure S2.

Clinical studies

Five patients (4 Male 1 Female), age (51 - 66 yo) with a recurrent high-grade glioma was scanned at 3T (Philips Healthcare, Cleveland, OH) using a 32 channel head coil. Multiple

FA data was acquired (TR=7.6 ms, TE=3.7 ms, FA=2°-20° in 2° increments, FOV=240×240 mm², ST=5 mm, matrix=96²) to produce a $T_{1\rho}$ map. Next, a multi-echo single-shot EPI acquisition (TR=1.5 s, TE1/TE2=7/31 ms, FOV=240×240 mm², ST=5 mm, matrix=96²) was performed before, during, and after administration of 0.1 mmol/kg Gd-DTPA at an infusion rate of 4 ml/s (followed by a saline flush). The scan duration was 7.5 minutes and followed all guidelines set by the Vanderbilt University Institutional Review Board. Measures of R_2^* and R_1 were calculated from the multi-echo data for the entire time-course (36). DSC-MRI perfusion maps were calculated from the R_2^* measurements and an automated measure of the AIF, using circular SVD-based deconvolution (38). Maps of K^{trans} , v_e and ADC (DW SE-ssEPI, TR=6s, TE=44ms, NEX=2, b=100,1000 s/mm²) were also computed. Voxel wise estimates of C_t and $TRATE$ were calculated using Eq. 4.

Statistical Analysis

Single variable and multivariable associations between cell density and $TRATE$ in animals were estimated using standard regression analysis. Statistical relationships between $TRATE$ and K^{trans} , ADC , and v_e were performed using both ROI and voxel-wise analysis. For ROI analysis, standard linear regression relationships between mean parameters for each animal or patient were used. Voxel-wise prediction of $TRATE$ by cell line, and K^{trans} , ADC , and v_e were conducted using analysis of covariance in a generalized linear model assuming a normal distribution, the identity link with an exchangeable covariance working model. Generalized estimating equations were used to account for intra-voxel correlation within animals or humans. Standard goodness-of-fit and residual analyses were conducted and found unremarkable.

Results

Fig.1 shows example FPFDM simulation results, demonstrating the main input and output parameters. Fig. 1A shows a 3D volume rendering of a sample tissue structure, which contains a 60% volume fraction of ellipsoids with an average radii of 15 μ m (note that only ¼ of the total number of cells are incorporated to aid in structure visualization) and a 6% vascular volume fraction with vessel sizes ranging from 5 μ m to 45 μ m. For illustrative purposes, the two compartment pharmacokinetic model described by Brix *et al* was used to create the C_e and C_p time curves (with clinically relevant input parameters) shown in Fig. 1B (39). Fig. 1C shows a representative 2D slice through the tissue structure along with magnetic field perturbations computed at three time points indicated in Fig. 1B. For time points near the peak of C_p , where C_e is very small, the field perturbation is dominated by the vascular structure, as indicated by the strong field perturbations surrounding the vessels. As C_e increases, the field perturbation around cells starts to emerge and dominates the vascular field perturbation at time points near CA equilibrium, as illustrated by the elevated field perturbations surrounding ellipsoids. The resulting R_2^* time curve is shown in Fig. 1D.

For CA concentration levels similar to tracer equilibrium and a range of relevant cellular and vascular volume fractions, Fig. 2A shows the computed R_2^* values for two tissue structures containing only vascular networks (volume fractions of 6% and 9%) and four tissue structures containing only packed ellipsoids (volume fractions ranging from 20% to

60%). At a χ value of $4\pi \times 0.2 \times 10^{-7}$ ([CA]=0.75 mM), corresponding to one fifth the peak susceptibility difference for a 0.1 mmol/kg dose of Gd-DTPA, the computed R_2^* values for the 45% and 65% cellular phantoms are 78% higher than that computed for the phantom containing a 9% vascular volume fraction. In general, this difference increases with increasing susceptibility difference between tissue compartments. Fig. 2B shows R_2^* values computed at five different tracer equilibrium concentrations for four different tissue structures with fixed cellular packing volume fraction of 55% and varying vascular volume fractions ranging from 3% to 12%. At a given tracer equilibrium concentration, the computed R_2^* values are approximately equivalent for all vascular volume fractions. Across vascular volume fractions, a maximum difference of only 4% exists between the computed R_2^* values, indicating that tissue vascularity has a negligible contribution to the computed R_2^* values at CA equilibrium, when compared to the effects of CA compartmentalized within the EES.

The dependence of R_2^* on cellular features (e.g. cell volume fraction and size) is shown in Fig. 3A. For a fixed cellular size, as cell volume fraction increases, the R_2^* first increases and then decreases, reaching a peak value between a cell volume fraction of 30% and 50%, depending on the cell size. In general, as the cell size increases the peak R_2^* shifts to higher cell volume fractions. The computation in Fig. 3A is carried out at a fixed vascular volume fraction of 6% and equilibrium CA concentration corresponding to $\chi = 4\pi \times 0.5 \times 10^{-7}$. For a given cell volume fraction, increasing the cell size induces larger R_2^* values. This indicates that, under these simulated conditions the range of tissue structures does not reach the static dephasing regime even for cell sizes near 20 μm . Supplementary Figure S1 shows the perturber size dependence of R_2^* for tissue structures with various cell volume fractions. These results show that the perturber size corresponding to the point where the diffusion independent regime for the gradient echo relaxation rate begins shifts to the right (larger perturber sizes) as cell volume fraction increases. Accordingly, at cellular volume fraction levels (50-60%) observed *in vivo* (40), the R_2^* retains its sensitivity to cell size. For instance a change in cell size from 5 μm to 20 μm results in a 25% increase in R_2^* for a 25% cell volume fraction but a 45% increase when the cell volume fraction is 50%.

Fig. 3B shows the dependence of R_2^* on tissue CA concentration for two tissue structures, both with 6% vascular volume fraction and 50% cellular volume fraction, but different cell sizes. As compared to R_2^* values computed for the tissue structure with smaller cell size (1 μm), the computed R_2^* values for the tissue structure built using larger cell size (20 μm) has higher values at each CA concentration levels, owing to its larger field perturbation and a smaller diffusion narrowing process.

The dose response of R_2^* shown in Fig. 3B was used to estimate the *TRATE* parameter using the slope of a linear regression fit. Fig. 3C shows the dependence of *TRATE* on cell size and volume fraction. For both tissue structures, *TRATE* increases with increasing cell volume fraction well up to 60%, unlike the R_2^* values, which peaked near at relatively low cell volume fraction. For a given cell density, *TRATE* is also shown to increase with cell size.

Experimental *TRATE* measurements for cell phantoms at various cell volume fractions are shown in Supplementary Figure S2. In general *TRATE* increases with increasing cell density for both cell lines. For a given cell density the *TRATE* values in MEL cells are higher than those found in HL-60 cells.

Example multi-echo DSC-MRI derived R_2^* and R_1 time curves for C6 glioblastoma, 9L gliosarcoma and normal tissue ROIs are illustrated in Fig. 4. The R_1 time curves in C6 and 9L tumors and normal tissue are different in both magnitude and shape, illustrating the differences between the CA kinetics within these tissues. The derived R_2^* time series in 9L tumors exhibited substantial and prolonged (out to 15 minutes) T_2^* leakage effects. In C6 tumors, these effects, while present, were less pronounced, as the R_2^* values plateau after three minutes. In normal tissue, the R_2^* values decrease to nearly pre-contrast levels immediately after the peak.

Figure 5 depicts example C6 and 9L, K^{trans} , v_e , *ADC* and *TRATE* maps. Within a given tumor, the *TRATE* values were spatially heterogeneous. In these examples, the *TRATE* values were also markedly higher in the 9L tumors as compared to those in the C6 tumors. The *TRATE* maps exhibited little visual similarity with the other parameters.

Supplementary Figure S3 displays representative voxel-wise relationships between *TRATE* and K^{trans} , v_e , and *ADC* corresponding to the median correlation coefficients across all animals. A weak correlation was observed between *TRATE* and K^{trans} ($r = 0.17$), v_e ($r = -0.065$), *ADC* ($r = -0.08$). Across all the rats, the voxel-wise correlation coefficients, shown in Table 1, between *TRATE* and K^{trans} , v_e , *ADC* were low (with a maximum $r = 0.54$) and diverse. A voxel-wise comparison of the parameters across all the animals revealed no significant association between *TRATE* and K^{trans} ($p=0.205$) or v_e ($p = 0.442$). There was a non-zero statistically significant association with *ADC* ($p=0.002$), which, in part, reflects the large number of observations (voxels) included in the analysis. However, with an R^2 value of only 0.22 there exists a considerable degree of variance in *TRATE* that is not accounted for in the *ADC* values.

Sample histologic images from H&E staining indicate that the C6 tumor cell density (Fig. 6A) is lower than that found in the 9L tumors (Fig. 6B). Cell nuclei count analysis shows on a group result that 9L tumors contain 30% more cells than C6 tumors (Fig. 7A). Consistent with this histology, *ADC* values in 9L tumors ($0.87 \times 10^{-3} \text{ mm}^2/\text{s}$) were significantly lower than those found in C6 tumors ($1.23 \times 10^{-3} \text{ mm}^2/\text{s}$) as shown in Fig. 7B. The *TRATE* values in 9L tumors were significantly higher ($24.5 \text{ mM}^{-1} \text{ s}^{-1}$) than those found in C6 tumors ($12.9 \text{ mM}^{-1} \text{ s}^{-1}$) as shown in Fig. 7C. Note that these values of *TRATE* are substantially larger than the range of T_2 relaxivity values for Gd-DTPA ($3.8\text{--}4.5 \text{ mM}^{-1} \text{ s}^{-1}$) measured in water and plasma solutions at 4.7T (35). Across animals H&E staining estimates of the C6 and 9L tumor cell volume fraction were $56.2 \pm 4.3 \%$ and $74.5 \pm 3.3 \%$, respectively. Cell sizes were estimated to be $5.02 \pm 0.6 \mu\text{m}$ and $8.4 \pm 1.7 \mu\text{m}$ for the C6 and 9L tumor cells, respectively.

Figure 8 shows the ROI linear regression analysis of *TRATE* and cell density across all animals. A significant correlation between *TRATE* and cell density ($R^2 = 0.44$, $p = 0.005$) is

observed when the tumor cell type is ignored. However, in a mixed linear regression analysis, which accounts for the two cell lines, there was no correlation between $TRATE$ and cell density ($p = 0.641$), indicating that, within a given cell type, the relationship between $TRATE$ and cell density is unknown.

Figure 9 shows example multi-echo DSC-MRI data acquired from a ROI in a high-grade glioma patient. Similar to the 9L tumors, the derived R_2^* time curves exhibit T_2^* leakage effects as shown in Fig. 9A. Immediately after the first pass, the R_2^* only decreases by 40% of the peak value and then slowly decreases over the course of minutes, consistent with kinetics expected for CA extravasation. In contrast, normal tissue R_2^* values decrease to nearly 85% of the peak value immediately after the peak and quickly plateau near pre-contrast levels. Example R_1 time curve computed using T_{10} map and the extrapolated dual-echo data for tumor ROI is shown in Fig. 9B.

Figure 9C shows example maps of the tumor CBF, CBV, v_e , ADC and $TRATE$. As was the case in the animal tumor models, $TRATE$ is heterogeneous across the tumor. The voxel-wise correlation coefficients, shown in Supplementary Table S1, between $TRATE$ and the other imaging parameters were low (with a maximum $r = 0.61$) and diverse. On a mean scale, $TRATE$ and ADC exhibited a linear and negative relationship ($r^2 = 0.81$, $p = 0.03$, $TRATE = 435.86 - 38.75 \times ADC \times 10^4$), but on a voxel-wise basis, across the five patients, $TRATE$ showed a statistically significant association with v_e ($p=0.003$, $TRATE = 20.880 + 4.626 \times v_e$). However, similar to the animal data, the voxel-wise correlation coefficients were low, as shown in Supplementary Table S1, and indicate that this association is very small.

Discussion

Previous reports have shown that T_2^* leakage effects influence DSC-MRI data and have suggested a link between their presence and tissue microstructure(14,15,27-29,41-43), but, to date, their biophysical basis is poorly characterized. In this study, we validate, for the first time, using simulations and *in vitro* and *in vivo* data, that T_2^* leakage effects, at CA equilibrium, are primarily influenced by cellular characteristics. We also propose a straightforward method to quantify these effects by calculating the $TRATE$ parameter, and show that it can be used to differentiate between brain tumors with histologically distinct cellular properties.

A key finding of the computational studies is that at tracer equilibrium the magnetic field perturbations are predominantly influenced by CA compartmentalization around cells. During the first pass of the agent the greatest field perturbation heterogeneity, as expected, is localized around blood vessels. However, at CA equilibrium, the tissue reduces to two effective compartments, the intracellular and extracellular space. Consequently, the cellular features primarily determine the induced field perturbation. Although the field perturbation created by individual cells might be weaker and fall faster than the field perturbation induced by vessels, given the high cell volume fraction of tissues, field perturbations induced by densely packed cells tends to be highly heterogeneous across the voxel, thereby influencing a large number of water protons. Consequently, susceptibility contrast induced

parameters, such as *TRATE*, computed long after the first pass of the CA, are expected to depend on the underlying cellular features.

Simulation results demonstrate that cellular size and volume fraction influence the computed R_2^* and *TRATE* values. For all simulated cell volume fractions, the R_2^* values were larger for tissue structures packed with larger cells compared to those with smaller cells. For a given cell size, the observed dependence of R_2^* on cell volume fraction (Fig. 3A) can be understood by examining the cell volume fraction induced changes to the computed local magnetic field perturbation (B) and the standard deviation of the magnetic field perturbation between the simulated grid points within the voxel (B_{std}). At low cell volume fractions (e.g. 20%) the addition of cells into the structure will increase both the B in the vicinity of the new cell and the B_{std} . At higher cell volume fractions (~35-45%), adding new cells increases B in their vicinity and the B_{std} will reach a peak. Beyond this peak, adding more cells will continue to increase the B , however, as the structure becomes more populated, a higher fraction of the protons will experience similar B values, causing the B_{std} to decrease. This observation is consistent with the theoretical treatment of the transverse relaxation rate taken to be proportional to the product of the mean square frequency deviation and the correlation time of the frequency fluctuation (43-45). For a fixed diffusion correlation time, the mean square frequency deviation, which is determined by the standard deviation of the field perturbation (B_{std}), determines the R_2^* . Furthermore, the results presented in Fig. 3A agree closely with previously reported R_2^* dependence on haematocrit measured *ex vivo* in blood samples (43). It is interesting to note that over a physiologic range of cell volume fractions that may be expected in viable tissue (e.g. 45% - 65%) the simulation results in Fig 3A indicate *TRATE* heterogeneity or contrast most likely reflects variations in cell size.

The results shown in Supplementary Figure S1 are consistent with previous reports of the characteristic perturber size dependency of relaxation rates. Furthermore, these computational results demonstrate the influence of cell density on the strength of the diffusion narrowing process. At fixed values of B_0 , χ and D , the effect of diffusion persists well into large cell sizes as the cell density increases. The presented simulation results are consistent with previous theoretical results based on the Anderson-Weiss mean field theory, where the underlying factors that contribute to transverse relaxation rate variations, including diffusive correlation time and variations in Larmor frequency due to field perturbations, were found to depend on the perturber volume fraction (45). Consequently, for high cell densities, the perturber size where R_2^* reaches a plateau occurs at relatively larger cell sizes as compared to lower cell density. This suggests that for physiologically relevant tumor cell volume fractions levels (50-60%) observed *in vivo* (40), DSC-MRI derived R_2^* values are highly sensitive to variations in tumor cell size (46).

The dependence of R_2^* on C_t exhibits a slight deviation from a linear relation (Fig. 3B). This is consistent with previous studies where a quadratic relation is observed at low CA concentration (13,47). However, using CA concentration values that would be expected at equilibrium, 0.1-0.5 mM, a linear regression fit yielded estimates of *TRATE* with regression values higher than 0.99. Unlike the R_2^* dependence on cell volume fraction, where the peak R_2^* occurs at relatively small v_i , the computed *TRATE* values increase with v_i beyond

60% and is expected to decrease for high v_i values (Fig. 3C). This shift of the peak to higher v_i values results from the quantification of C_t as the sum of individual compartment CA concentration weighted by the corresponding volume fractions ($C_t = v_e C_e + v_p C_p$). For a given equilibrium CA concentration (C_{eq}) and v_p values, the calculated C_t values, which are equivalent to $(1-v_i)C_{eq}$, are lower for high cell density structures as compared to those with lower cell density, thereby increasing *TRATE*. Although a more stringent systematic and realistic simulation study on the relation between *TRATE* and various physical, physiological and pulse sequence parameters is warranted, these initial computational results demonstrate that *TRATE* provides a reasonable range of sensitivity to *in vivo* cellular volume fraction levels (40).

As described above, Sourbron *et al* recently proposed T_2^* - relaxivity contrast imaging (RCI) as a means to characterize intra- and extravascular CA relaxivities *in vivo* (16). In general, *TRATE* imaging may be considered a subset of RCI as it focuses only on measuring CA relaxivity at tracer equilibrium rather than over the entire time course. Practically, the assessment of *TRATE* is computationally simpler as it does not rely upon multiple post-processing steps, including the extraction of parameters from a DCE-MRI two-compartment exchange model and the reliable separation C_p and C_e time curves. This later step highlights a key difference between RCI and *TRATE*. Whereas RCI aims to separate the intra- and extravascular CA relaxivities, *TRATE*, by definition, is the effective *tissue* CA relaxivity and, thus, only requires measuring the tissue CA concentration, which can be directly computed from the R_1 time course. In the context of a DSC-MRI brain tumor study, the assessment of *TRATE* does not involve additional kinetic modeling and may provide a clinically feasible approach for characterizing tumor cellularity.

In vitro cell phantom experiments (Supplementary Figure S2) further demonstrated the dependency of *TRATE* on cell size and density. While the dependence of *TRATE* on cell density is apparent from Fig. S2, for a given cell density the difference in *TRATE* values between the MEL and HL-60 cell lines is likely a result of changes in cellular size and shape.

Both pre-clinical and clinical multi-echo DSC-MRI datasets exhibited substantial T_2^* leakage effects well after the first pass of the CA. Though traditional DSC-MRI exams in brain tumors are typically no more than three minutes, this study confirm the presence of considerable T_2^* effects even out to ten minutes following CA injection. With single-echo acquisition methods, the detection of these T_2^* effects is confounded by simultaneous and competing changes in T_1 , which is likely why they are only now being considered as a source of new tissue contrast with MRI. It is also important to note that the *TRATE* values in the brain tumors studied here were much greater than the microscopic T_2 relaxivity of Gd-DTPA, verifying that mesoscopic susceptibility changes across a voxel are the source of the T_2^* leakage effects.

Histology and *ADC* measurements indicate that 9L tumors have a higher cell density than that found in C6 tumors. Histological analysis also revealed that the 9L cells were large than C6 cells. Consistent with the computational results showing that *TRATE* increases with increasing cell volume fraction and cell size, the *TRATE* values in 9L tumors were

significantly higher than those in C6 tumors. A significant correlation between *TRATE* and cell density was observed when the tumor cell type was ignored (Fig. 8). However, the lack of correlation between *TRATE* and cell density within each cell line indicates that other cellular features, in addition to cell density variations (e.g. size, shape, spatial distribution) are driving the differences in *TRATE*. Moreover this lack of correlation may reflect that the respective cell densities for the C6 and 9L tumor cells are near the peak of the R_2^* versus cell volume fraction relationship (Fig. 3A) and thereby less sensitive to variations in cell density.

In addition to the dissimilar *TRATE* values between the C6 and 9L tumor types, there was little to no spatial correlation (Supplementary Fig. S3), between *TRATE* and *ADC*, K^{trans} or v_e , indicating that the variance in the *TRATE* maps is not accounted for by these parameters and that *TRATE* is potentially reporting on unique microstructural properties of the tumor tissue. Similar results were found in the human parameter comparisons. The only exception was the ROI mean *TRATE* versus *ADC* comparison, which yielded a significant correlation between the parameters. Consistent with the computational and *in vitro* findings, tumors with low *ADC* values, potentially reflecting those with high cellularity, also exhibited high *TRATE* values. Additional studies with more patients is needed to explore this potential relationship, however, because no such correlation was found for the voxel-wise comparison. The dependence of *ADC* on cellular properties is well established (48). As shown here, *TRATE* is sensitive to cell density but also upon other physical or physiological parameters that could alter the susceptibility induced transverse relaxation rates, such as, diffusion, cell membrane permeability, cell size, shape and overall organization (13,29,42,49-52). While *TRATE* and *ADC* depend on similar microstructural features their sensitivities to each will likely differ due to the differences in their underlying contrast mechanisms, which may explain the low voxel-wise correlation coefficients between *TRATE* and *ADC*. Further studies are warranted to investigate the specific differences between the sensitivity of *TRATE* and *ADC* measurements to tissue cytoarchitecture.

From Fig. 4 it is clear that 9L and C6 tumors exhibit marked differences in their R_1 time courses, which is indicative of differences in vascular hemodynamics, tissue compartment sizes and permeability. Thus, the sensitivity of R_2^* measurements alone to tumor microstructure, even at CA equilibrium, is likely confounded by heterogeneous and dynamic changes in the tissue CA concentration. Because estimates of *TRATE* incorporate measures of the tissue CA concentration, the effect of these differences is minimized. *TRATE*, therefore, is instead influenced by local CA distribution within the EES. This sensitivity is in contrast to other perfusion-based imaging metrics such as PSR, which represents a complex combination of tissue microstructure and hemodynamic effects including blood flow, blood volume, vascular permeability, cell volume fraction and cellular geometry. Fortunately, the use of multiple-echo DSC-MRI, permits the separation of many of these factors through estimates of R_1 and R_2^* , enabling the isolation of tissue geometrical factors (e.g cell density and distribution). In addition, the acquisition of multiple echoes and calculation of absolute tissue relaxation rates likely makes the method for estimating *TRATE* less sensitive to pulse sequence parameters when compared to metrics such as PSR (53). Validation of this point, however, is the subject of future analysis.

Though pulse sequence parameters, such as echo time, may have nominal influence on the estimate of *TRATE*, the time, after CA injection, during which the measurement is made could be significant. Estimation of *TRATE* relies on the assumption that the distribution of CA is in a state of equilibrium between the vascular and extravascular extracellular space. With the use of a two-compartment pharmacokinetic modeling (39) it can be shown that, for a range of physiological parameters relevant to brain cancer, CA equilibrium occurs approximately five to seven minutes after CA injection. For this reason, estimation of *TRATE* requires a moderately longer DSC-MRI acquisition time. Additionally, physiological phenomena such as tissue necrosis could also influence *TRATE* measurements. In the case of tumor necrosis, which often occurs in both animal and human brain tumors, diffusion of CA into the necrotic region can occur, resulting in continuously increasing R_1 (54). The estimate of *TRATE* in these regions may be confounded if CA equilibrium is never reached.

As previously indicated, there do exist limitations on the method for calculating *TRATE*. These limitations, however, are mainly focused on the tissue being analyzed and more specifically its vascular permeability. To reiterate, estimations of *TRATE* require CA extravasation out of the vasculature. Though computing a threshold for the amount of CA extravasation required is beyond the scope of the work presented here, the change in R_1 after contrast injection should be many times greater than the standard deviation of the baseline R_1 to avoid contributions from noise. Future studies will seek to establish voxel-wise criteria on when *TRATE* can be reliably measured and its reproducibility.

In addition to studies aiming to further characterize the biophysical basis of *TRATE*, there are numerous opportunities to explore the clinical role of *TRATE* measurements. Given the previously shown potential of PSR to differentiate between lymphomas, gliomas and brain metastasis based on the presence or absence of T_2^* leakage effects (27), it is likely that *TRATE* would show similar differences while at the same time providing a quantitative and pulse sequence independent measure. The sensitivity of *TRATE* to cytoarchitecture suggests its potential role to assess treatment-induced cytotoxicity, similar to the current use of functional diffusion mapping (55). Finally, although *TRATE* was assessed here using a DSC-MRI acquisition, it could just as easily be estimated as part of a multi-echo DCE-MRI study or even a multi-echo, post-CA injection steady-state exam. The use of these methods would enable the estimation of *TRATE*, and therefore cytoarchitectural features, at higher resolution and in any tissue in or outside the brain using clinically available pulse sequences.

Conclusion

DSC-MRI is commonly used to assess the vascular and hemodynamic status of brain tumors. When acquired with multi-echo pulse sequences and pre-contrast T_1 maps the studies described herein show that, by leveraging T_2^* leakage effects, tumor cellular features can also be interrogated through the parameter *TRATE*. The sensitivity of *TRATE* to tissue cytoarchitecture indicate that it could potentially serve as a unique structural signature or “trait” of different types of cancers and may be useful as a biomarker of cancer aggressiveness and early treatment response. Results in pre-clinical and clinical brain tumors indicate that *TRATE* provides unique information not present in DCE-MRI kinetic parameters or DWI based *ADC* maps. Further computational and *in vivo* studies are needed

to systematically characterize the dependency of *TRATE* on microstructure (e.g. cellular orientation heterogeneity, shape, spatial distribution), establishing its reproducibility and sensitivity to cell density variations in other tumor types and during the course of treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Example FPFDM simulation

(A) Sample tissue structure composed of ellipsoids packed around fractal tree based vascular network. (B) Simulated C_p and C_e curves derived using 2-compartment model. (C) Example 2D map through the tissue structure along with magnetic field perturbation computed at three different time points, showing the increasing contribution of the cells after the first pass. (D) The time evolution of the R_2^* computed at $B_0 = 3 \text{ T}$ using the C_p and C_e curves in (B).

Figure 2. Comparison of vascular and extravascular induced R_2^*

(A) A plot of the dose response of R_2^* for four cellular and two vascular tissue structures. The computed R_2^* values for cellular structures are substantially higher than those for the vascular structures. (B) A plot of R_2^* dependence on tracer equilibrium concentration levels, for four tissue structures of fixed cellular structure but different vascular networks. At a given equilibrium concentration level the difference between the computed R_2^* values is negligible, which indicates that at equilibrium the cellular features rather than the vascular differences drive the R_2^* values.

Figure 3. Dependence of R_2^* and TRATE on cell size and volume fraction

(A) A plot of R_2^* dependence on cell volume fraction and size. For a given cell volume fraction R_2^* is larger for structures with 20 μ m ellipsoids as compared to that with 10 μ m ellipsoids. For a given cell size R_2^* is sensitive to changes in cell volume fraction reaching a peak value approximately between 35% and 45%. (B) The linear response of R_2^* to changes in tissue CA concentration for two tissue structures with different cell sizes. (C) The influence of cell volume fraction and size on TRATE. Unlike R_2^* which peaks near 40% cell volume fraction, TRATE increases up to 60% cell volume fraction.

Figure 4. Example *in vivo* R_2^* and R_1 time curves

Example dual-echo DSC-MRI derived R_2^* (A), and R_1 (B) time curves for the C6 and 9L rat brain tumor ROIs along with a representative normal tissue. The 9L tumors exhibit elevated R_2^* values that persist until the end of the scan. The magnitude of these effects was much lower in C6 tumors. The R_1 time curves for C6 and 9L tumor are markedly different indicating their unique pharmacokinetic characteristics.

Figure 5. Example *in vivo* parameter maps

A comparison of $TRATE$ maps with K^{trans} , v_e , ADC maps and anatomical post Gd-DTPA T_1 weighted images in the C6 and 9L tumors. Visually, $TRATE$ maps were dissimilar to the other imaging parameters.

Figure 6. Example histological images

Representative H&E images for the C6 (A), and 9L (B) tumors, shows high cell density in 9L tumors.

Figure 7. *In vivo* parameter estimates

(A) A group analysis of the cell nuclei count shows that 9L tumor types contain approximately 30% more cells than the C6 tumors. (B) The average ADC values in 9L tumors are significantly lower than those found in C6 tumors. (C) The average *TRATE* values in 9L tumors were significantly higher than those found in C6 tumors.

Figure 8. ROI based relationship between *TRATE* and cell density

A linear regression analysis independent of cell type result in a significant correlation between *TRATE* and cell density ($R^2 = 0.44$, $p = 0.005$) (bold line). A linear regression analysis, which accounts for the two cell lines, demonstrates no correlation between *TRATE* and cell density ($p = 0.641$) (dotted lines).

Figure 9. Example clinical dual-echo derived R_2^* and R_1 time curves and parameter maps
 Representative dual-echo DSC-MRI derived R_2^* and R_1 in a patient with a recurrent high-grade glioma. (A) The tumor ROI R_2^* time curve exhibits prolonged T_2^* leakage effects, whereas in normal tissue the R_2^* values, after the first pass, decrease rapidly to pre-contrast levels. Example R_1 time curve computed using T_{10} map and the dual-echo data for tumor ROI is shown in (B). (C) Example maps of tumor blood volume, blood flow, v_e , ADC, TRATE along with a post-contrast T_1 weighted anatomical image in a glioma patient. The TRATE map is heterogeneous across the tumor ROI and visually dissimilar to the other parameter maps.

Table 1

Voxel-wise Pearson's correlation coefficient (r) between $TRATE$ and ADC , K^{trans} and v_e for each animal included in the study.

Rat #	ADC	K^{trans}	v_e
1	-0.34	-0.49	-0.25
2	0.046	0.33	0.23
3	-0.18	0.01	-0.01
4	-0.12	0.18	-0.05
5	-0.39	0.30	-0.18
6	0.05	0.17	-0.11
7	-0.27	-0.06	-0.02
8	0.06	-0.19	-0.14
9	-0.27	0.22	-0.42
10	0.13	0.17	-0.08
11	0.31	0.54	0.51
12	-0.04	0.09	-0.03
13	-0.33	0.38	-0.15
14	0.01	0.25	-0.00